

Rapid Testing of Non-Dairy and Mixed Dairy Beverages Using the 3M™ Microbial Luminescence System (MLS, formerly Cogent)

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Introduction:

Adenosine Tri-Phosphate (ATP) testing systems have been used for several years in the rapid testing for commercial sterility in Ultra High Temperature (UHT) and Extended Shelf Life (ESL) dairy products. These ATP testing systems share a common approach to the detection of contamination in UHT products. In essence, the product to be tested is pre-incubated for 48-72 hours to allow single microbes to grow exponentially. The samples are then treated with an ATPase to remove non-microbial ATP prior to the detection of microbial ATP using Luciferase to produce a bioluminescent signal. The differentiation between microbial ATP and bovine ATP depends on the accessibility of each by the ATPase. The cell walls of microbes protect the microbial ATP from the ATPase. In contrast the thin plasma membrane of the bovine cells – if they survived UHT treatment – and fat globules which may harbor bovine ATP are easily ruptured using a non-ionic surfactant.

With the increasing prevalence of juices and teas there are new applications for ATP testing systems. In these matrices, however, the cell walls of the plant cells protect the product ATP from the ATPase making it significantly more difficult to reduce the product ATP to a level where low levels of microbial contamination can be detected.

The data presented here demonstrates that the inclusion of a proprietary mix of non-ionic surfactants can improve the degradation of plant ATP by the ATPase step and allows detection of contamination in various juice and tea products. In addition, the improved ATPase reagent mix retains its ability to detect microbial contamination in dairy products indicating that it may be used as a universal reagent for both dairy and non-dairy UHT sterility testing.

Materials and Methods:

3M™ Microbial Luminescence Screening Instrument (MLSII), 3M™ MLS Dairy Kit reagents, and the 3M™ MLS Juice Kit were obtained from 3M Microbiology. Various dairy and non-dairy UHT products were sourced from local supermarkets. Microbial strains were sourced directly from ATCC or indirectly through Microbiologics, Inc.

UHT products were aseptically divided into aliquots of 2.5 mL in plastic culture tubes. Samples were dosed with high (<5 x 10³), medium (<5 x 10²), or low (<50) number of bacteria diluted in Butterfield's Buffer. Samples were incubated at 32°C for time periods stated. At the times specified, samples were vortexed and 50 microliters of sample was placed in the wells of 96 well plate and analyzed using the 3M MLSII System and reagents from 3M. Microbial concentrations were estimated using 3M™ Petrifilm™ Plates using dilutions and standard methods or by spotting dilutions onto the middle and four quadrants of a 3M Petrifilm Plate.

Results:

Background Reduction

Thirty uninoculated samples of a variety of UHT treated products were analyzed using both the 3M MLS Dairy Kit and the 3M MLS Juice Kit. The mean of the 30 samples is displayed below. Where the mean was skewed heavily by sporadic false positives, a mean calculated on the results excluding the false positives is shown in parenthesis.

Product	Dairy Kit		Juice Kit	
	Average RLU	Average RLU	Average RLU	Average RLU
Low Fat Chocolate Milk	10.4	13.6		
Vanilla Soy Milk	12.1	13.2		
Almond Milk	12.3	13.8		
Spiced Tea Concentrate	198.2	26.0		
Ready-To-Drink Tea with Lemon	6789.2	18.4		
Grapefruit Juice	69299.2	23.9		
Carrot/Orange Juice Blend	496.1 (52.3)	79.9 (26.5)		
Mango Nectar	72.8 (42.5)	43 (25.7)		
Strawberry Banana Juice	148.3 (43.2)	65.4 (23.5)		
Apple Juice	40.3	30.8		
Tomato Soup	22.9	22.1		
Sweet Potato Bisque	24.8	24.3		

The number of false positives was also affected by the juice kit in some matrices, but not in the Mango Nectar:

Product	Dairy Kit False Positives	Juice Kit False Positives
Carrot/Orange Juice Blend	11	2
Mango Nectar	4	4
Strawberry Banana Juice	4	1

Discussion:

The 3M MLS Juice Kit has been shown to reduce the backgrounds of UHT tea, juice, and soup products in the data presented here. In addition to reducing the background, the 3M MLS Juice kit also demonstrated an ability to reduce (though not eliminate) the incidence of high values likely to be attributed to larger particles of plant material suspended in the opaque juice samples such as grapefruit juice, carrot/orange juice, and Mango Nectar. However, the high incidence of high value wells (4/30) in the mango nectar demonstrates the need for either additional development of the reagent or an alteration of the ATPase incubation parameters.

Detection of Microbes

Dairy, soy, almond milk, tea, and juice samples were spiked with nothing (control), *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, or *Candida albicans* and incubated for 18 hours or 48 hours. While the tea and juice products were also dosed with the bacterial cultures in the same manner as the dairy products, the microbes did not survive in these matrices as evidenced by an absence of colonies on 3M Petrifilm Plates from the 48 hr pre-incubated samples.

18-24 Hour Pre-Incubation

	Chocolate Milk		Vanilla Soy		Almond Milk		Spiced Tea Conc.	Tea with Lemon	Grapefruit Juice	Carrot/Orange Juice Mix	Mango Nectar	Strawberry Banana	Apple
	Dairy	Juice	Dairy	Juice	Dairy	Juice	Juice	Juice	Juice	Juice	Juice	Juice	Juice
<i>E. coli</i>	+	+	+	+	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>P. aeruginosa</i>	+	+	+	+	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>B. subtilis</i>	+	+/-	+	+	+	+/-	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>S. aureus</i>	+	+	+	+	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>S. cerevisiae</i>	+	+/-	+	+	+	+	+/-	+/-	+	+	+	+	+
<i>C. albicans</i>	+	+	+	+	+	+	+	+/-	+	+	+	+	+

48 Hour Pre-Incubation

	Chocolate Milk		Vanilla Soy		Almond Milk		Spiced Tea Conc.	Tea with Lemon	Grapefruit Juice	Carrot/Orange Juice Mix	Mango Nectar	Strawberry Banana	Apple
	Dairy	Juice	Dairy	Juice	Dairy	Juice	Juice	Juice	Juice	Juice	Juice	Juice	Juice
<i>E. coli</i>	+/-	+	+	+	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>P. aeruginosa</i>	+	+	+	+	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>B. subtilis</i>	+	+	+	+	+	+/-	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>S. aureus</i>	+	+	+	+	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>S. cerevisiae</i>	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>C. albicans</i>	+	+/-	+	+	+	+	+	+	+	+	+	+	+

* All samples for both 24 hr pre-incubation and 48 hr pre-incubation time points were positive for growth on Petrifilm Plates.

Discussion:

The 3M MLS Juice Kit successfully detected contamination of the juice and tea products with yeasts in most samples after 24 hour pre-incubation and in every sample after 48 hour pre-incubation. Note, however, that the sample volumes were low and exposure to oxygen was high relative to standard packaging.

The 3M MLS Juice Kit was fairly successful in detecting a wide range of contaminants in dairy products. The chocolate milk samples that failed at 24 hour pre-incubation were at the lower limit of detection (10³ CFU/mL). The *B. subtilis* sample in almond milk was similarly very slow growing, though was at a level (10⁴ CFU/mL) that should consistently give a positive result.

Conclusions:

The 3M MLS Juice Kit indicates promising results in juice, tea and dairy products when incubated at 24 and 48 hours for a wide range of contaminants. Further development of the ATPase incubation parameters and additional validation is planned by 3M.

